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Characterization of Anti-Hepatitis B Surface Antigen Monoclonal Antibodies

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Summary. 17 monoclonal antibodies generated against purified hepatitis B surface antigen (HBsAg), subtype *ayw*, were characterized by solid-phase radioimmunoassays. Eleven of these antibodies had specificity against the group-specific *a* determinant of HBsAg, two demonstrated antibody activity against the *w* HBsAg subtype, one against human serum albumin, and three against human IgG. All monoclonal antibodies were of the IgG class.

Hepatitis B surface antigen (HBsAg) is associated with and represents the envelope of the 42-nm particle of hepatitis B virus (HBV). Purified preparations of this glycosylated lipoprotein are physically heterogeneous and consist of at least seven polypeptides ranging in molecular weight from 22,000 to 97,000 [1–4]. Serologically, HBsAg has one group-specific determinant called *a* and two sets of mutually exclusive subtype determinants: *d* or *y* and *w* or *r*. Combinations of the *a* determinant with the subtype determinants result in four major serotypes: *adw*, *ayw*, *adr*, and *ayr* [5–7]. All HBsAg-derived polypeptides contain in their structure the group-specific and at least one of the subtype-specific antigenic determinants, and these determinants are exposed on the surface of the HBsAg particle [8].

With the advent of hybridomas [9], it has become possible to generate monoclonal antibodies that react specifically with either the group-specific or subtype antigenic determinants of HBsAg. Several laboratories have previously reported the production and characterization of monoclonal antibodies against HBsAg [10–13]. In this present work, we characterized a library of monoclonal antibodies obtained in our laboratory by fusing NS-1 myeloma cells to spleen cells from mice immunized with the *ayw* subtype of HBsAg.

HBsAg was purified from three pools of human plasma positive, respectively, for *adw*, *ayw*, and *adr* subtypes of HBsAg as previously described [14]. Only the purified *ayw* subtype

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Table I. Reactivity of hybridomas

Clone	S/N ratio[1]						Specificity	Immunoglobulin composition
	HBsAg			HSA	HuIgG	NHS		
	adw	*ayw*	*adr*					
A-1	13.3	9.3	12.0	0.2	1.8	1.8	anti-*a*	γ_1, λ
A-2	24.3	5.5	6.3	0.7	0.6	1.2	anti-*a*	γ_1, κ
A-3	2.8	2.3	2.4	1.1	1.3	1.6	anti-*a*	γ_1, λ
A-4	48.8	10.3	26.6	0.9	0.7	0.9	anti-*a*	γ_1, κ
A-5	4.8	6.8	14.1	1.3	1.8	0.8	anti-*a*	γ_2, κ
A-6	11.9	4.5	8.6	0.9	1.6	1.2	anti-*a*	γ_1, κ
A-7	11.6	4.7	8.9	1.1	0.9	0.7	anti-*a*	γ_2, κ
A-8	34.3	9.7	7.9	0.8	0.9	0.8	anti-*a*	γ_3, κ
A-9	38.5	90.6	28.8	1.3	0.7	1.4	anti-*a*	γ_1, κ
A-10	92.9	26.4	45.1	2.0	1.5	1.1	anti-*a*	γ_1, κ
A-11	19.6	18.8	21.3	0.8	0.6	0.5	anti-*a*	γ_1, λ
W-1	5.3	9.4	1.8	1.1	1.3	1.4	anti-*w*	γ_1, λ
W-2	8.8	7.9	1.2	0.7	0.9	0.8	anti-*w*	γ_1, λ
HSA-1	87.0	26.0	50.3	149.5	1.8	31.5	anti-HSA	γ_1, κ
HuIgG-1	4.6	3.8	4.4	1.5	4.1	19.1	anti-IgG	γ_2, κ
HuIgG-2	4.0	3.3	4.2	1.3	4.0	18.9	anti-IgG	γ_3, κ
HuIgG-3	35.6	24.3	28.6	1.0	31.5	22.4	anti-IgG	γ_2, κ

[1] Ratio calculated by dividing the mean cpm of each hybridoma supernatant fluid (S) by the mean cpm determined for a supernatant fluid obtained from NS-1 myeloma cells (N).

of HBsAg was used to generate monoclonal antibodies. The production of monoclonal antibodies, fusion and cloning in soft agar were performed according to *Kennett* [15]. The screening for anti-HBsAg secreting clones was done by a micro solid-phase radioimmunoassay (micro-SPRIA) using HBsAg/*ayw* as coating antigen and ^{125}I-labeled goat anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.) as the second antibody [16]. The specificity of 17 monoclonal antibodies generated by two separate fusions was further characterized using a similar micro-SPRIA with each of the following as coating antigens: HBsAg subtypes *adw*, *adr* and *ayw*, purified human serum albumin (HSA), human IgG (HuIgG), and normal human serum (NHS). The immunoglobulin (Ig) heavy chain class and light (L) chain isotype were determined for each supernatant fluid by an Ig class-specific radioimmunoassay that has been previously described [17].

Eleven of the 17 monoclonal antibodies reacted with the three HBsAg subtypes, yet failed to bind either HSA, HuIgG, or NHS; thus, these antibodies specifically reacted with the *a* cross-reacting group determinant(s) of HBsAg (table I).

During the screening process we noted that individual anti-*a* hybridomas had different patterns of reactivity with the three HBsAg subtype preparations. As shown in table I, several hybridomas reacted equally well with all three subtypes (clones A-1, A-3, A-11),

whereas others demonstrated a preferential reaction for one or two subtypes (A-5 with HBsAg/*adr*; A-9 with HBsAg/*ayw*; A-4, A-6 and A-7 with HBsAg/*adw* and *adr*; A-2, A-8 and A-10 with HBsAg/*adw*). This observation suggested that different monoclonal specificities were obtained against the group *a* determinant, because the mice were immunized with the same HBsAg/*ayw* preparation for both fusions and all the clones were tested against the same HBsAg preparations. To rule out the possibility that individual hybrid cell lines were producing two or more antibody specificities, each preparation was recloned in soft agar. The recloned cell lines secreted antibody with reactivity patterns similar to that of the parent cell population. These results are in agreement with the heterogeneity of the *a* determinant that has been previously reported [18].

Two monoclonal antibodies reacted equally well with the *ayw* and *adw* subtypes of HBsAg while failing to bind *adr* and NHS components (table I). Thus, these antibody preparations were specifically designated as anti-*w* reactions.

It is noteworthy that one monoclonal antibody preparation recognized HSA and three reacted specifically with HuIgG antigenic determinants (table I). Since an HBsAg-associated polypeptide of molecular weight 68,000 has been shown to be immunochemically similar to HSA [19], it was not surprising to obtain a monoclonal antibody that recognized HSA. However, the characterization of monoclonal antibodies which reacted with HuIgG suggested that the preparation of HBsAg used to immunize mice prior to fusion may have been contaminated with IgG as a result of the purification procedure. The presence of NHS proteins in association with purified HBsAg preparations has been a continuous problem

Table II. Characteristics of other anti-HBs hybridomas

Hybridoma	S/N ratio[1]					
	HBsAg			HSA	HuIgG	NHS
	adw	*ayw*	*adr*			
Y-HSA	1.6	3.4	1.6	2.6	1.6	1.1
A-HuIgG-1	126.3	46.7	79.9	1.8	4.2	0.9
A-HuIgG-2	21.1	20.9	12.4	1.7	3.0	1.0
A-HSA	37.9	9.9	7.5	3.9	0.9	0.9
W-HSA	3.8	11.6	1.7	4.1	1.5	1.1

[1] See footnote, table I.

and matter of controversy. Some authors suggested that human proteins such as albumin, apolipoprotein C and the γ chain of IgG are integral parts of HBsAg particles [20]. The presence of low concentrations of immune complexes in carriers has been suggested as an alternative possibility for contamination of HBsAg preparations with IgG. However, other authors were unable to confirm these associations [21]. Different methods of purification, including low pH, enzyme digestion, and immunoadsorption, were developed to circumvent this problem, but trace amounts of NHS components are routinely noted [for review see 22].

Supernatant fluids from other anti-HBsAg hybridoma cells demonstrated differential reactivities with respect to the three HBsAg subtype preparations and also bound to HSA or HuIgG (table II). Included with these hybridomas were some that produced antibody against the *y* subtype determinant. It was thought that such hybridoma cells were not of a single antibody-producing cell type. These are currently being recloned and tested to insure the production of monoclonal antibodies.

Of the 17 monoclonal antibodies character-

ized, no IgM or IgA antibodies were detected. 12 were κ L chain bearing, while five contained λ L chains (table I). The γ chain subclass breakdown was as follows: 11 of the monoclonals were γ_1, and 6 were of the γ_3 subclass (table II).

This panel of monoclonal antibodies generated to HBsAg will be useful in determining the antigenic structure and complexity of the group- and type-specific HBsAg determinants. They will also be invaluable tools for serological analysis of synthetic polypeptides that are currently under investigation as possible vaccines for prevention of HBV infections [23–26].

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